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### Authors

Chen, Qian  
Francis, Ellen  
Hu, Gang  
et al.

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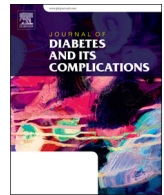
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# Metabolomic profiling of women with gestational diabetes mellitus and their offspring: Review of metabolomics studies

Qian Chen <sup>a,b</sup>, Ellen Francis <sup>c</sup>, Gang Hu <sup>a</sup>, Liwei Chen <sup>c,\*</sup>

<sup>a</sup> Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA, United States

<sup>b</sup> Guangdong Provincial Key Laboratory of Food, Nutrition and Health, Department of Nutrition, School of Public Health, Sun Yat-sen University, Guangdong, China

<sup>c</sup> Department of Public Health Sciences, Clemson University, Clemson, SC, United States

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## ABSTRACT

**Background:** Gestational diabetes mellitus (GDM) reflects an increased risk of developing type 2 diabetes (T2D) after pregnancy in women. Offspring born to mothers with GDM are at an elevated risk of obesity and T2D at a young age. Currently, there are lack of ways for identifying women in early pregnancy who are at risk of developing GDM. As a result, both mothers and fetus are not treated until late in the second trimester when GDM is diagnosed. The recent advance in metabolomics, a new approach of systematic investigation of the metabolites, provides an opportunity for early detection of GDM, and classifying the risk of subsequent chronic diseases among women and their offspring.

**Methods:** We reviewed the literatures published in the past 20 years on studies using high-throughput metabolomics technologies to investigate women with GDM and their offspring.

**Conclusions:** Despite the inconsistent results, previous studies have identified biomarkers that involved in specific metabolite groups and several pathways, including amino acid metabolism, steroid hormone biosynthesis, glycerophospholipid metabolism, and fatty acid metabolism. However, most studies have small sample sizes. Further research is warranted to determine if metabolomics will result in new indicators for the diagnosis, management, and prognosis of GDM and related complications.

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## 1. Introduction

Gestational diabetes mellitus (GDM), one of most common pregnancy complications, is defined as any degree of glucose intolerance with onset or first recognition during pregnancy.<sup>1</sup> The prevalence of GDM varies worldwide, ranging from 1 to 14% of all pregnancies depending on diagnostic criteria and study population.<sup>2</sup> Recent data have shown a significant increase in the prevalence of GDM among women of various ethnic/racial backgrounds and in different geographic regions.<sup>3–7</sup> The increase in prevalence of GDM is likely to continue as a rise in obesity rates for girls and women of reproductive age has been observed globally.

### 1.1. Diagnosis of GDM

The diagnosis of GDM relies on detecting elevated plasma glucose, often in the late of the second trimester; however, there currently is no

global consensus on the diagnostic criteria. In 2010, The International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommended a “one-step” approach, in which all pregnant women without known diabetes (type 1 or 2) receive a 75 g 2-hour oral glucose tolerance test (OGTT) between 24 and 28 weeks gestation.<sup>8</sup> In 2013, the National Institutes of Health (NIH) expert panel recommended the “two-step” approach, in which pregnant women who screen positive after a 50 g 1-hour OGTT, take a subsequent 100 g 3-hour OGTT between 24 and 28 weeks gestation.<sup>9</sup> These approaches also differ on whether two abnormal values are required, and what diagnostic cut-off values should be used. Given that there are data to support both approaches, in 2014 the American Diabetes Association (ADA) recommended both options for diagnosing GDM.<sup>1</sup>

### 1.2. Health consequences for women with GDM and their offspring

Although most women return to a normal glucose status after delivery, 20% of women with GDM develop impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) during 6–12 weeks postpartum.<sup>10,11</sup> More importantly, women with GDM are at increased risk of developing of type 2 diabetes (T2D) later in life. The increased risk of T2D among women with GDM has been documented in different populations and countries. On average, the risk of developing of T2D is

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\* Corresponding author at: Department of Public Health Sciences, Clemson University, Clemson, SC 29634, United States.

E-mail addresses: [chenq52@mail2.sysu.edu.cn](mailto:chenq52@mail2.sysu.edu.cn) (Q. Chen), [ecfranc@g.clemson.edu](mailto:ecfranc@g.clemson.edu) (E. Francis), [Gang.Hu@pbrc.edu](mailto:Gang.Hu@pbrc.edu) (G. Hu), [liweic@clemson.edu](mailto:liweic@clemson.edu) (L. Chen).

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7.4 times greater for women with GDM compared to women without GDM.<sup>10</sup> A large retrospective cohort study in Australia demonstrated the risk of developing diabetes increased with time from women's index pregnancy: the cumulative incidence of diabetes was 2.6% at 2 years, 8.1% at 5 years, 17.3% at 10 years, and 25.8% at 15 years after diagnosis of GDM.<sup>12</sup> Recent data have also indicated that independent of T2D or obesity, women with GDM might be at an increased risk of hypertension,<sup>13</sup> metabolic disorders<sup>14</sup> and even cardiovascular disease (CVD) events.<sup>15</sup> In addition, emerging evidence tends to suggest that women with GDM represent a population who are more likely to develop CVD at a relatively younger age.<sup>16</sup>

GDM also has profound impacts on the offspring's health, both short- and long-term. Biologically, maternal glucose can cross the placenta but maternal insulin cannot. In response to the increased glucose load, the fetal pancreas increases insulin production, which in turn, promotes fetal growth and adiposity development.<sup>17</sup> There is a growing body of data that supports a positive association between maternal hyperglycemia and children's risk of health problems, including obesity, diabetes, insulin resistance, IGT, T2D, CVD risk factors,<sup>18–21</sup> and autism.<sup>22</sup> The available evidence suggests the higher risk of obesity and T2D in offspring of GDM mothers is likely due to the intrauterine environment which is independent of shared socioeconomic, lifestyle and genetic factors within families.<sup>23,24</sup> However, the molecular pathways underlying such associations remain unclear.

### 1.3. Metabolic features of GDM

GDM is a significant risk factor for development of T2D and CVD in women and offspring. Therefore, identifying women at high risk of GDM and understanding the metabolic changes in the early life of their offspring has important public health and clinical significance. During the past several decades, a number of risk factors for GDM have been identified, including advanced maternal age, family history of diabetes, pre-pregnancy obesity, and multiple pregnancies.<sup>25</sup> However, the etiology and pathogenesis of GDM remains unclear.

Pregnancy is accompanied by numerous metabolic changes in women, one of which is a substantial decrease in insulin sensitivity. Pregnancy-induced insulin resistance progressively develops in the second and third trimesters and is thought to be secondary to increases in maternal adiposity, as well as increases in placental and other hormones (e.g. human placental lactogen, placental growth hormone, progesterone, estradiol, leptin, cortisol, prolactin, human chorionic gonadotropin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and other inflammatory mediators).<sup>26–28</sup> Pregnant women have an increased insulin secretion compared to a non-gravid state.<sup>29</sup> In some women their  $\beta$ -cell function cannot compensate for the increased insulin requirements in late pregnancy, and as a result develop GDM or hyperglycemia.<sup>30</sup> Why these women have inadequate  $\beta$ -cell compensation is not well known. GDM may be caused by a complex set of interactions between genetic and environmental factors, triggered by a series of metabolic challenges during pregnancy (e.g. promoting adipose tissue accumulation in middle gestation and developing insulin resistance in late pregnancy).

Although the etiology and pathogenesis of GDM is not fully understood, the majority of women with GDM appear to have  $\beta$ -cell dysfunction that occurs in the background of chronic insulin resistance. To date, only a few potential biochemical mediators of chronic insulin resistance associated with GDM have been investigated. It is likely that there is not a single pathway or etiology that contributes to the development of GDM. In addition, GDM may influence fetal organ development and growth through maternal-placental-fetal glucose/insulin physiology and these may result in a downstream effect on the fetal brain and peripheral systems. This complexity presents a challenge for completely understanding the spectrum of molecular mechanisms associated with maternal diabetes and how they impact physiological and metabolic changes in offspring. The examination of one or several targeted

molecules utilizing traditional methods has failed to detect the relevant underlying molecular changes.

### 1.4. Overview of metabolomic

Progress in this research may be facilitated by recent developments in technologies for comprehensive metabolic analysis, known as “metabolomics”.<sup>31</sup> This approach allows for large-scale simultaneous analysis of numerous metabolites, and detects subtle changes in the metabolic network in human bio-fluids or tissues. In addition, metabolomics can provide certain advantages relative to other “omics” technologies (genomics, transcriptomics, or proteomics) because it measures molecular phenotypes that are the net results of other ‘omics’, therefore providing the most integrated profile of biological status.

The use of metabolomics for better understanding the etiology and pathogenesis of disease consists of two sequential steps. The first step is an experimental technology, based on liquid or gas chromatography–mass spectrometry (MS), or proton (<sup>1</sup>H) nuclear magnetic resonance (NMR) spectroscopy. MS provides higher sensitivity and the opportunity of optimized targeted analysis, and NMR offers an overview of mode metabolites, structural information, and higher reproducibility. Using these new technologies, metabolomics can investigate the complete set of metabolites or low-molecular-weight intermediates (molecular weight < 1000) in a given biological sample. The second step is to perform multivariate data analysis to determine differences in the metabolic profiles of individuals with certain health conditions/diseases versus healthy controls. In brief, metabolomics is a comprehensive method for qualitative and semi-quantitative analyses of metabolites in human bio-fluids or tissues. It has been rapidly used in the early detection of diseases, measuring biological responses to treatments/interventions, identifying the underlying pathways between risk factors and diseases, and discovering novel biomarkers associated with certain health stages/conditions.

### 1.5. Metabolomics in GDM

Metabolomics has been used to analyze metabolic profiles and identify novel biomarkers associated with insulin resistance and T2D. The metabolic groups have been reported included carbohydrate metabolites (e.g. glucose and fructose), lipid metabolites (e.g. phospholipids, sphingomyelins, and triglycerides), and amino acid metabolites (branched-chain amino acids, aromatic amino acids, glycine, and glutamine).<sup>32–35</sup> The pathophysiological changes that occur in T2D and GDM are similar and use of this new technology may aid our understanding of the etiology and pathogenesis of GDM. Furthermore, metabolomics may increase our ability to identify early predictors of GDM or classify the risk of subsequent CVD among women and their offspring. These innovations call for a thorough review of what has been reported in this area of literature to inform future directions for research, summarize what has been found, and elucidate any challenges in the field.

## 2. Literature research

We searched PubMed from January 2007 to April 2017 for studies using high-throughput metabolomic technologies (i.e. MS or NMR) to investigate women with GDM and their offspring. Key-words used included “diabetes,” “gestational,” “gestational diabetes,” “metabolome,” “metabolomics,” “metabolic disease,” “metabolic syndrome,” “impaired glucose,” “HOMA-IR,” “HOMA-beta,” “insulin resistance,” “overweight,” “obesity,” “body mass index,” “BMI,” “body weight”, and “adiposity” (see Appendix A). Additionally, hand searching of the references cited in identified articles was performed. Two authors (QC & EF) independently searched the literature, reviewed the studies, and extracted the data. Then two senior author (LC and GH) double checked the literature search and the information extracted from each included article.

The findings of the review are organized in the following manner: 1) metabolic profile of mothers in early pregnancy (prior to the diagnoses of GDM) that predict GDM; 2) metabolic profile of mothers with a diagnosis of GDM compared to those without at the time of diagnosis; 3) metabolic profile at the postpartum of mothers who had experienced GDM compared to those who did not; 4) the changes in metabolic profile over time of women with GDM as compared to women without, and 5) metabolic profile of offspring who born to mothers who experienced GDM and those who did not.

### 2.1. Metabolomics to predict GDM (bio-specimens collected before the diagnosis of GDM)

The early identification of women who are at risk of developing GDM is important since early treatment/interventions can be introduced to reduce the adverse impacts of hyperglycemia for both mothers and offspring. We identified 9 studies, all with nested case-control design, that compared the metabolic profile (before the diagnosis of GDM) of women who later developed GDM to women who did not. Of them, blood samples (plasma/serum) were analyzed in five studies, urine samples were analyzed in four studies, and amniotic fluids were analyzed in two studies (some studies analyzed two types of bio-specimens). We provided a summary table (Table 1) of all studies that included in this review.

For women who later developed GDM compared to those who did not, 5 studies that analyzed *blood samples (plasma/serum)* reported significant increases of itaconic acid, *cis*-aconitate acid,<sup>36</sup> anthranilic acid, alanine, glutamate, allantoin, serine,<sup>37</sup> total fatty acids, myristic acid, palmitic acid, palmitoleic acid, eicosapentaenoic acids,<sup>38</sup> flavonoids (sesaminol 2-O-triglucoside, and tricin 7-neohesperidoside)<sup>39</sup>; and significant decreases in creatinine,<sup>37</sup> phospholipids (unsaturated), (2E)-14-hydroxytetradec-2-enoic acid or its isomer, (2E,13R)-13-hydroxytetradec-2-enoic acid, 2,15-dihydroxy-pentadecanoic acid (or its isomers), (7R,8S,9Z,12Z,15Z)-7,8-dihydroxy-9,12,15-octadecatrienoic acid,<sup>39</sup> trimethylamine-N-oxide and betaine.<sup>40</sup>

Among the 4 studies that analyzed *urine samples*, a significant increase was reported for women with GDM compared to controls in choline,<sup>40–42</sup> N-methyl-nicotinamide,<sup>40,41</sup> glucose, xylose,<sup>41</sup> 3-hydroxyisovalerate, 2-hydroxyisobutyrate, N-methyl-2-pyridone-5-carboxamide,<sup>40</sup> and citrate<sup>43</sup>; and in one study a significant decreases of 4-hydroxyphenylacetate and hippurate were reported.<sup>41</sup> Two studies measured amniotic fluid<sup>42,44</sup> and both reported increases in glucose and decreases in glutamate. One study has also identified decreases in glycine, proline, serine, taurine, acetate, formate, creatinine, and glycerophosphocholine.<sup>44</sup>

In summary, studies on the metabolic profile of women in early pregnancy to predict risk of GDM has yielded minimally consistent findings; with studies reporting decreases in blood creatinine, trimethylamine-N-oxide, and betaine<sup>37,40</sup>; and increases of urine N-methyl-nicotinamide,<sup>40,41</sup> and urine choline.<sup>40–42</sup> Two studies also found increases of amniotic fluid glucose, and decreases of amniotic fluid glutamate.<sup>42,44</sup>

### 2.2. Metabolomics to distinguish profiles of women with GDM (bio-specimens collected after or simultaneous to GDM diagnosis)

Identifying metabolic differences between women who are diagnosed with GDM compared to those who are not during the third trimester may lead to better understanding of the associated complications, as well as potential influences on the metabolic development of fetus. We identified 12 studies that compared the metabolic profile of women with GDM to women without GDM simultaneous or after diagnosis in late pregnancy. These studies were mostly case-control in design with only 4 studies utilizing a nested case-control design.<sup>38,39,43,45</sup> A summary of the findings and specific metabolic differences are reported in Table 1.

*Blood samples (plasma/serum)* were analyzed in 9 studies comparing metabolic profiles of women with GDM to controls. Increases and decreases of metabolites within the same structural and functional class were reported for women with GDM. Most consistently, increases in amino acids were reported in 5 studies,<sup>45–49</sup> and increases in fatty acids were reported in four studies.<sup>38,48–50</sup> Additional metabolites that were found to be significantly increased in women with GDM were prostanoid acid, sesaminol 2-O-triglucoside, tricin 7-neohesperidoside, dihydro-12-oxo-15-phytoenoic acid,<sup>39</sup> acetylcarnitines,<sup>48,49</sup> bile acids,<sup>51</sup> ketones,<sup>47,48</sup> creatinine, carbohydrate (primary glucose),<sup>47</sup> and other lipids and organic acids. Decreases in phospholipids, (2E)-14-hydroxytetradec-2-enoic acid (or its isomer), (2E,13R)-13-hydroxytetradec-2-enoic acid, 2,15-dihydroxy-pentadecanoic acid (or its isomers), (7R,8S,9Z,12Z,15Z)-7,8-dihydroxy-9,12,15-octadecatrienoic acid, 11 $\alpha$ ,20,26-trihydroxyecdysone,<sup>39</sup> glycerophospholipids,<sup>45,48,49</sup> 1,5-anhydroglucitol,<sup>47</sup> and gluconic acids<sup>45,48</sup> were also reported. Similarly, decreases in amino acids,<sup>45,46,48,49</sup> and decreases in fatty acids were also reported.<sup>45,48,49</sup>

Of the 3 studies that analyzed urine samples, one found citrate as a significantly different biomarkers to distinguish women with GDM from non-GDM women,<sup>43</sup> while 2 studies reported increases in amino acids,<sup>48,52</sup> with 1<sup>48</sup> also reporting a decrease in carnitine.

The analysis of hair samples was conducted in 1 study<sup>53</sup> and increases in adipic acid were found.

In summary, the metabolic profile of women with GDM compared to controls has been found to have significant increases and decreases in specific metabolites. Despite the differences reported in the aforementioned studies, there is little consistency in the specific metabolites that were significant, and often the direction of change is contrary within a metabolic structural or functional class.

### 2.3. Metabolomic profiles after the pregnancy (bio-specimens collected in postpartum or later)

Differences in the metabolomic profile after the pregnancy are likely associated with the long-term complications of GDM and could be used as screening tools for predicting the risk of future complications. Such information is also important to help developing optimal risk screening strategies and interventions to reduce women's future risk of diabetes and CVD.

We only identified 3 studies that examined postpartum bio-specimens, of which *blood (serum/plasma)* was collected at three days postpartum by one study,<sup>45</sup> and at twenty-two months following birth by another.<sup>54</sup> The 3rd study analyzed *urine samples* collected at 10–16 weeks postpartum.<sup>43</sup> The serum/plasma metabolic profile of women who had experienced GDM compared to women without GDM showed differences in levels of amino acids in two studies.<sup>45,54</sup> Additional differences in phospholipids, acylcarnitines, fatty acids, and diglycerides were also reported.<sup>54</sup> No significant biomarkers to distinguish GDM women and non-GDM women in the analysis of postpartum urine.<sup>43</sup>

Reconciling the inconsistent results is challenging as all three studies had substantial timing differences of sample collection, and type of mass-spectrometer used. Likely, the type of specimen may explain the inconsistent, given both studies examining blood found significant results, whereas analysis of urine was not.

### 2.4. Metabolomic profiles over time during the pregnancy (bio-specimen collected longitudinally during pregnancy or postpartum)

Studies examining the changes in metabolites over time for pregnant women with GDM as compared to women without GDM during pregnancy and postpartum would provide the most valuable data for understanding the etiology and pathogenesis of the GDM. However, only 4 studies were identified that collected bio-specimens longitudinally.<sup>38,39,43,45</sup> Among 4 studies three studies only made

**Table 1**

Summary of results by time of specimen collection from metabolomic studies investigating profiles of women with and without GDM.

| Author, country, year   | Study design & methods  | Sample collection time | Bio-specimens                | # of GDM/ non-GDM     | Diagnostic criteria | Metabolites in GDM group compared to control group  |  |
|---|---|------------------------|------------------------------|-----------------------|---------------------|---|--|
|   |   |                        |                              |                       |                     | Increased   | Decreased  |
| <i>Bio-specimens collected priori to GDM diagnosis</i>                |   |                        |                              |                       |                     |   |  |
| Chen, US, 2010  | Nested case-control (targeted on FAs, GCMS)                       | 16 weeks GA            | Serum (fasting)              | 49/80 impaired GCT/98 | C & C               | FAs (myristic acid, palmitic acid, palmitoleic, and eicosapentaenoic acids)   | None   |
| Graça, Portugal, 2010   | Nested case-control (untargeted, NMR)                             | 14–25 weeks GA         | Amniotic fluid (non-fasting) | 27/82                 | N/A                 | Glucose   | Amino acids (glutamate, glycine, proline, serine, taurine), organic acids (acetate, formate), creatinine, glycerophosphocholine  |
| Diaz, Portugal, 2011  | Nested case-control, untargeted metabolite profiling (NMR)        | 14–25 weeks GA         | Plasma (non-fasting)         | 14/20                 | N/A                 | None  | Trimethylamine- <i>N</i> -oxide and betaine  |
|   |   |                        | Urine (non-fasting)          | 29/25                 |                     | Choline, 3-hydroxyisovalerate, 2-hydroxyisobutyrate, <i>N</i> -methyl-2-pyridone-5-carboxamide, <i>N</i> -methyl-nicotinamide   | None   |
| Graça, Portugal, 2012   | Nested case-control, untargeted metabolite profiling (LCMS & NMR) | 15–25 weeks GA         | Amniotic fluid (non-fasting) | 23/26                 | N/A                 | Glucose   | Amino acids (glutamate)  |
|   |   |                        | Urine (non-fasting)          | 20/21                 |                     | Choline   | None   |
| Sachse, Norway, 2012  | Nested case-control, untargeted metabolite profiling (NMR)        | 8–20 weeks GA          | Urine (fasting)              | 79/530                | WHO or IADPSG       | Citrate   | None   |
| Diaz, Portugal, 2013  | Nested case-control, untargeted metabolite profiling (NMR)        | 14–26 weeks GA         | Urine (non-fasting)          | 42/84                 | N/A                 | Choline, glucose, <i>N</i> -methyl-nicotinamide, xylose   | 4-Hydroxyphenylacetat, hippurate   |
| De Seymour, New Zealand, 2014   | Nested case-control (untargeted, GCMS)                            | 20 weeks GA            | Serum (N/A)                  | 22/26                 | N/A                 | Itaconic acid, <i>cis</i> -aconitate acid   | None   |
| Bentley-Lewis, US, 2015   | Nested case-control (untargeted, LCMS)                            | <12 weeks GA           | Serum (fasting)              | 96/96                 | C & C               | Anthranilic acid, allantoin, amino acids (alanine, glutamate, and serine)   | Creatinine   |
| Law, China, 2017  | Nested case-control (untargeted, LCMS)                            | 11–14 weeks GA         | Plasma (fasting)             | 27/34                 | IADPSG              | Sesaminol 2- <i>O</i> -triglucoside, tricin 7-neohesperidoside  | Phospholipids (unsaturated and/or chemically modified), (2 <i>E</i> )-14-hydroxytetradec-2-enoic acid or its isomer, (2 <i>E</i> ,13 <i>R</i> )-13-hydroxytetradec-2-enoic acid, 2,15-dihydroxy-pentadecanoic acid (or its isomers), (7 <i>R</i> ,8 <i>S</i> ,9 <i>Z</i> ,12 <i>Z</i> ,15 <i>Z</i> )-7,8-dihydroxy-9,12,15-octadecatrienoic acid   |
| <i>Bio-specimens collected after or simultaneous to GDM diagnosis</i> |   |                        |                              |                       |                     |   |  |
| Chen US, 2010   | Nested case-control (targeted on FAs, GCMS)                       | 30 weeks GA            | Serum (fasting)              | 49/80 impaired GCT/98 | C & C               | FAs (palmitic acid, palmitoleic acid, oleic acid, linoleic acid (18:2), linolenic acid (18:3), arachidonic acid (20:4), eicosapentaenoic acid, and docosahexaenoic acids)         | None   |
| Sachse, Norway, 2012  | Nested case-control (untargeted, NMR)                             | 26–30 weeks GA         | Urine (fasting)              | 79/530                | WHO or IADPSG       | Citrate   | None   |
| Dudzik, Poland, 2014  | Case-control (untargeted, GCMS/LCMS/CEMS)                         | 22–28 weeks GA         | Plasma (fasting)             | 20/20                 | WHO-1998            | Phosphatidylethanolamines (38:6, 36:5), Phosphatidylcholines (38:1, 40:3), acetylcarnitine, FAs (linoleic acid), glycerol, 3-hydroxybutyrate, 2-hydroxybutyrate, and fumaric acid | Glycerophospholipids (e.g. lysophosphatidylethanolamines (LPE with 16:0, 18:0, 18:2, 20:0, 20:1, 20:2, 22:4 and 22:6 chains); lysophosphatidylcholines (LPC with 16:0; 18:0, 18:1, 18:2, 18:3, 20:3, 20:4, 20:5 acyl chains); lysophosphatidylinositol (LPI 20:4); lysophosphatidylserine (LPS with 20:0); lysophosphatidic acid (LPA with 18:2); lipoxin C4), bile acids (trihydroxy-cholestanoyl taurine; taurothiocholic acid glucuronide), araquidonate and docosahexaenoic acid methyl esters, glycerophosphocholine, creatinine, pyruvic acid, amino acids |



Table 1 (continued)

| Author, country, year               | Study design & methods   | Sample collection time | Bio-specimens    | # of GDM/non-GDM                                    | Diagnostic criteria                    | Metabolites in GDM group compared to control group   |  |
|-------------------------------------|--|------------------------|------------------|---|--|--|--|
|                                     |  |                        |                  |   |  | Increased  | Decreased  |
|                                     |  |                        | Urine (fasting)  |   |  | Amino acids (histidine, glutamine, phenylalanine, tryptophan and cystine)  | (L-tryptophan, glycine, L-glutamic acid), FAs (lauric acid) Carnitine  |
| Prentice, US, 2014                  | Case-control (untargeted GCMS/LCMS)                            | 24–28 weeks GA         | Plasma (fasting) | 24/24   | NIH                                    | FAs (primary polyunsaturated fatty acids: the largest increased is 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF))   | None   |
| Scholtens, multiple countries, 2014 | Case-control (targeted on amino acids and untargeted, MS/GCMS) | 24–32 weeks GA         | Serum (fasting)  | 67/50 (<10th percentile end of FPG) from HAPO study | IADPSG                                 | Amino acids (alanine, proline, and branched-chain amino acids), carbohydrates (primary glucose), lipids (palmitic acid, lauric acid, heptadecanoic acid, $\beta$ -tocopherol), ketone bodies ( $\beta$ -hydroxybutyric acid, acetoacetic acid), and organic acids (pyruvic acid, $\alpha$ -ketoglutaric acid, 2-hydroxybutyric acid, isocitric acid, and creatinine) | 1,5-Anhydroglucitol, mannitol and other hexitols, $\beta$ -sitosterol  |
| Lorenzo, Poland, 2015               | Case-control (targeted on amino acids, GCMS)                   | 22–28 weeks GA         | Urine (fasting)  | 20/20   | WHO-1998                               | D-Phenylalanine  | None   |
| Hajduk, Poland, 2015                | Case-control (targeted on amino acids, LCMS)                   | 24–28 weeks GA         | Plasma           | 18/13   | Polish Gynecological Society Standards | L-Citrulline   | L-Asparagine, L-valine, and L-ornithine  |
| He, China 2016                      | Case-control (untargeted, GCMS)                                | 24–28 weeks GA         | Hair             | 47/47   | IADPSG                                 | Adipic acid  | None   |
| Lehmann, Germany, 2015              | Case-control (targeted, GCMS/LCMS)                             | 24–27 weeks GA         | Plasma (fasting) | 9/15  | IADPSG                                 | Acetylcarnitine, amino acids (leucine), FAs (oleate)   | Glycerophospholipids, amino acid (glycine), lysophosphatidylcholines, FAs (C22:4, C22:5)   |
| Gao, China, 2016                    | Case-control (targeted on bile acid, LCMS)                     | ~28 weeks GA           | Serum (fasting)  | 38/27   | IADPSG                                 | Bile acids (1 trihydroxy unconjugated ( $\beta$ -muricholic acid), 2 dihydroxy conjugated (glycohyodeoxycholic acid and taurohyodeoxycholic acid) and 5 sulfated (dehydro-1S, mono-2S+ mono-3S, mono-4S, and mono-5S))   | None   |
| Liu, China, 2016                    | Nested case-control (untargeted, LCMS)                         | 28–36 weeks GA         | Serum (fasting)  | 12/10   | IADPSG                                 | 1-Methyladenosine, homovanillic acid, <i>trans</i> -3-octenedioic acid, glucosamine, lysophosphatidylcholines (LPC with 16:1, 20:2), 3-methylthiopropionic acid, L-lactic acid, amino acid (L-tyrosine), L-2-hydroxyglutaric acid, and phosphorylcholine   | Gluconolactone, serinyl-glycine, lysophosphatidylcholines (LPC with 20:4), 3-methylhistidine, 2-oxo-4-methylthiobutanoic acid, sphingomyelin (SM with d18:0/18:0), indoleacetaldehyde, phosphatidylethanolamines (PE with 14:1/20:0), 3-oxooctadecanoic acid, amino acid (glycine), pyridoxal, FAs (capric acid, stearic acid), 3-methylhistidine, and arabinonic acid |
| Law, China, 2017                    | Nested case-control (untargeted, LCMS)                         | 23–27 & 29–33 weeks GA | Plasma (fasting) | 27/34   | IADPSG                                 | Prostanoic acid, sesaminol 2-O-triglucoside, tricin 7-neohesperidoside, and dihydro-12-oxo-15-phytoenoic acid  | Phospholipids (unsaturated and/or chemically modified), (2E)-14-hydroxytetradec-2-enoic acid or its isomer, (2E,13R)-13-hydroxytetradec-   |

(continued on next page)

Table 1 (continued)

| Author, country, year                     | Study design & methods                 | Sample collection time | Bio-specimens   | # of GDM/ non-GDM                    | Diagnostic criteria | Metabolites in GDM group compared to control group   |   |
|---|--|------------------------|-----------------|--------------------------------------|---------------------|--|---|
|   |  |                        |                 |                                      |                     | Increased  | Decreased   |
|   |  |                        |                 |                                      |                     |  | 2-enoic acid, 2,15-dihydroxy-pentadecanoic acid (or its isomers), (7R,8S,9Z,12Z,15Z)-7,8-dihydroxy-9,12,15-octadecatrienoic acid, and 11α,20,26-trihydroxyecdysone                            |
| <i>Bio-specimens collected postpartum</i> |  |                        |                 |                                      |                     |  |   |
| Sachse, Norway, 2012                      | Nested case-control (untargeted, NMR)  | 10–16 weeks PP         | Urine (fasting) | 79/530                               | WHO                 | None   | None  |
| Anderson, UK, 2014                        | Case-control (untargeted, LCMS)        | 22 months PP           | Serum (fasting) | 18/39 (hyperglycemia but not GDM)/43 | WHO                 | Phospholipids (e.g. lysophosphatidylcholine (LPC with 20:1), lysophosphatidylglycine (LPG with 18:1), phosphatidylserine (PS with 30:7) acylcarnitines, FAs, amino acids, bile acids, and diglycerides | Phospholipids (e.g. lysophosphatidylcholines (LPC (10:1; 16:2)), lysophosphatidylethanolamine (LPE with 18:1), phosphatidylinositol (PI with 36:3), phosphatidylcholines (PC with 34:0; 38:0) |
| Liu, China, 2016                          | Nested case-control (untargeted, LCMS) | Within 3 days PP       | Serum (fasting) | 12/10                                | IADPSG              | L-Carnitine and creatine   | Amino acid (L-lysine), vitamin A, ribothymidine, corticosterone, lysophosphatidylethanolamine (PE with 22:6/0:0), 17-hydroxyprogesterone, and 1, 4-beta-D-glucan                              |

Note for all abbreviations: GCT, glucose challenge test; WHO, World Health Organization; IADPSG, International Association of Diabetes and Pregnancy Study Groups; GA, gestation age; PP, postpartum; GCMS, gas chromatography/mass spectrometry; LCMS, liquid chromatography/mass spectrometry; CEMS, capillary electrophoresis/mass spectrometry; NMR, nuclear magnetic resonance; C&C, Carpenter-Coustan criteria; FA, fatty acids; FPG fasting plasma glucose; HAPO Study the Hyperglycemia and Adverse Pregnancy Outcome Study.

cross-sectional comparisons (results have been summarized in previous sections and can be found in Table 1).<sup>38,43,45</sup> Cross-sectional comparisons provide insight into differences between GDM and non-GDM women at different time points during pregnancy, but do not allow for comparisons of the trend in metabolic profile change throughout gestation.

Only one recent study comparing women with GDM to women without GDM identified changes in the metabolic profile over time.<sup>39</sup> In this study, plasma samples were collected during the first, second, and third trimesters. The primary finding was that polyunsaturated (or chemically modified) phospholipids had an increasing trend in all women from the first to third trimester, but women with GDM had consistently lower levels as compared to the women without GDM – with the smallest difference observed at 23–27 weeks gestation.<sup>39</sup> Significantly higher levels of sesaminol 2-O-triglucoside and tricin 7-neohesperidoside, and lower levels of (2E)-14-hydroxytetradec-2-enoic acid (or its isomer) and (7R,8S,9Z,12Z,15Z)-7,8-dihydroxy-9,12,15-octadecatrienoic acid were also found in women with GDM throughout the pregnancy. In late pregnancy, significant differences of dihydro-12-oxo-15-phytoenoic acid, and 11 $\alpha$ ,20,26-trihydroxyecdysone, and prostanoid acid levels between GDM and non-GDM women were found.<sup>39</sup>

### 2.5. Metabolomic profiles of offspring of women who had GDM

Studies comparing the metabolic profile of offspring whose mothers had GDM to mothers who did not are extremely limited (Table 2). Two studies were identified in this area: one study examined newborns' meconium metabolome<sup>55</sup> and reported changes in lipid, amino acid, and purine metabolism for babies born to mothers with GDM; the other study examined cord blood<sup>56</sup> and reported increased levels of amino acids, pyruvate, histidine, alanine, methionine, arginine, lysine, hypoxanthine, lipoprotein and lipids, as well as lower levels of glucose. However, whether these metabolic differences are associated with the

development of obesity and diabetes in offspring of mothers who had GDM remains uncertain.

### 3. Summary and the future of research

Currently, there are a lack of tools with high sensitivity and high specificity capable of identifying women in early pregnancy who are at risk of developing GDM. As a result, both mothers and fetus are not treated until late in the second trimester when GDM is diagnosed based on current criteria. The uncontrolled metabolic changes during early pregnancy in women who later develop GDM are likely to have adverse consequences for fetal development, and for the long-term health for the offspring. Although high-throughput metabolomics is thought to aid our understanding of the etiology and pathogenesis of GDM, the current review outlines the complexity of drawing conclusions from current metabolomics research in GDM.

Many of the studies reviewed found significant differences in amino acids & related metabolites, fatty acids & related metabolites, phospholipids, and bile acids between women with GDM and without GDM. However, results are highly inconsistencies regarding the direction and significance of specific metabolites across studies. Findings from one study have not been replicated by another study. There are multiple possible explanations for such highly inconsistent results. First, studies in this area employed different bio-specimens. Of the studies included in this review, fifteen analyzed blood (plasma/serum), seven analyzed urine, two analyzed amniotic fluids, and one analyzed hair samples. Second, whether participants were fasting at time of biospecimen collection was inconsistent (Table 1). Third, the instruments and technologies used to analyze biospecimens varied across the studies. Fourth, GDM diagnosis criteria are different among studies. Lastly, age and ethnic/racial background of the study population are likely to contribute to the inconsistent findings.

Despite the inconsistent results from the literature, identified biomarkers from the reviewed metabolomics research are involved in specific metabolite groups and several important pathways,

**Table 2**

Summary of results from metabolomic studies investigating profiles of offspring born to women with and without GDM.

| Author, country, year | Study design                    | # of GDM/non-GDM | Offspring age | Diagnostic criteria | Bio-specimens    | Metabolites in GDM group compared to control group  |   |
|-----------------------|---------------------------------|------------------|---------------|---------------------|------------------|---|---|
|                       |                                 |                  |               |                     |                  | Increased   | Decreased   |
| Dani, Italy, 2014     | Case-control (untargeted, NMR)  | 30/40            | Newborn       | IADPSG              | Cord blood serum | Pyruvate, histidine, alanine, valine, methionine, arginine, lysine, hypoxanthine, lipoprotein and lipid   | Glucose   |
| Peng, China, 2015     | Case-control (untargeted, LCMS) | 142/197          | Newborn       | WHO                 | Meconium         | Amino acids derivative (argininosuccinic acid, tetrahydrodipicolinate); purine alkaloid metabolites (methyladenosine, methylxanthine, dimethyluric acid); bile acid derivative (taurodeoxycholic acid); DHAP (8:0); polyphenol host metabolites (methylepicatechin) | Amino acids derivative (hydroxyindoleacetyl glycine); purine alkaloid metabolites (methylguanosine; uric acid); bile acid derivative (glycocholic acid); fatty acid derivative (oxotrihydroxyleukotrienin B4); polyphenol host metabolites (vanilloylglycine) |
|                       |                                 |                  |               |                     | Urine            | Uric acid, uridine  | Estrone   |

Note for all abbreviations: WHO, World Health Organization; IADPSG, International Association of Diabetes and Pregnancy Study Groups; GCMS, gas chromatography/mass spectrometry; LCMS, liquid chromatography/mass spectrometry; CEMS, capillary electrophoresis/mass spectrometry; NMR, nuclear magnetic resonance; C&C, Carpenter-Coustan criteria; FA, fatty acids.

including amino acid metabolism, steroid hormone biosynthesis, glycerophospholipid metabolism, and fatty acid metabolism. For example, amino acids (particularly branched chain amino acids, BCAAs) have been consistently associated with insulin resistance and pancreatic  $\beta$ -cell dysfunction. Since 1969, a prominent BCAA signature of insulin resistance has been observed in normoglycemic adults, obese, prediabetes and diabetes population.<sup>32,57,58</sup> Several important metabolic pathways underlying these associations have been proposed. Recent studies found that elevated circulating BCAA might lead to excess trans-endothelial fatty acid transport into skeletal muscle, promote lipid accumulation and blunt insulin signaling via secretion of 3-hydroxyisobutyrate.<sup>59</sup> BCAAs play a key role in activating the pathway of the mammalian target of rapamycin complex-1 (mTORC1) involved in T2D, and the pathways of other metabolic disorders pathways.<sup>60</sup> This activation results in inducing a set of anabolic responses, including protein synthesis, cell growth, lipid synthesis, and, most importantly, insulin resistance.<sup>61</sup>

Although there are commonalities between the pathogenesis of T2D and GDM, and these disorders may share the previously mentioned metabolic pathway, the associations reported between amino acids and GDM in the current review were contrary or insignificant. Two studies observed that BCAAs were elevated in GDM women at 28 weeks gestation and 22 months postpartum,<sup>47,54</sup> one study reported decreased L-valine in GDM women at 24–28 weeks gestation,<sup>46</sup> and numerous studies found no significant differences.<sup>36,37,42,43,52,53</sup> The inconsistency findings in GDM are likely caused by the differences in genetic background, dietary intakes, and samples collected at different time points and environmental impacts.

The studies reviewed reported a series of lipid levels to be different between GDM and non-GDM women. Briefly, most studies examining pre- or post-diagnosis GDM cases, found fatty acids to be elevated,<sup>38,48–50,54</sup> while glycerophospholipids to be decreased<sup>44,45,48,49,54</sup> compared with healthy pregnancy controls. The mechanism of elevated maternal circulating fatty acids and GDM may be explained by increased insulin resistance and impaired  $\beta$ -cell function via accumulation of toxic intracellular lipid derivatives, oxidative stress, inflammation, and mitochondrial dysfunction.<sup>38,50,62,63</sup> The observations of decreased circulating glycerophospholipids (most prominent in lysoglycerophospholipids) during pregnancy<sup>39,45,48</sup> and postpartum,<sup>45,54</sup> are in line with findings from studies of T2D.<sup>32</sup> The alignment with studies of T2D suggests that the pathogenesis of GDM might be related to  $\beta$ -cell dysfunction,<sup>48</sup> alteration in enzymatic activities (e.g. lower levels of cytosolic calcium-dependent phospholipase-A2 isoform),<sup>64</sup> and an unbalanced pro-inflammatory versus anti-inflammatory ratio.<sup>48</sup>

It is worthy to note that the marked increase of glucose in amniotic fluid sample<sup>42,44</sup> and choline in urine samples<sup>40–42</sup> during the first and second trimester are possibly an early sign of hyperglycemia. Future studies are needed to confirm such results in different populations.

Huynh et al published the first high quality review of metabolomics studies of GDM in 2014.<sup>65</sup> However, the current review includes more recent studies published after February 2014. In addition, we reviewed studies by the timing of bio-specimen collection (e.g. before or after diagnosis of GDM, or postpartum), and reported studies that investigated the metabolic profile of offspring of mothers who had GDM in the index pregnancy. This review provided updates on the application of metabolomics in GDM epidemiological and clinical studies.

Several research gaps were identified by this review. Most studies were small in sample size, with the largest study including only 79 GDM cases and 530 non-GDM controls.<sup>43</sup> Few studies included information on dietary intake, which would have a significant influence on an individual's metabolites levels in blood, urine, or other tissue, particularly for amino acids and fatty acids and their related metabolites. Studies investigating the metabolic profiles of offspring born to GDM mothers are scarce, with only two studies identified in this review and only including newborns. Finally, the statistical methods used to compare metabolic profiles and identify significant differences between groups were highly inconsistent. Developing new computational tools to facilitate the analysis and interpretation of complex metabolomics data sets is highly needed.

From an epidemiological perspective, the use of metabolomics as a novel strategy for identifying GDM and improving maternal and infant outcomes requires refinement. The clinical implication for utilizing metabolomics to inform diagnosis, management, and prognosis of GDM and related complications is plausible, but as of yet there remains a need for further improvement in consensus of methodology before any informative conclusions can be drawn. In summary, future studies should investigate: 1) the appropriate bio-specimens that should be collected, 2) the optimal timing for collecting bio-specimens, 3) the adequate sample size, and 4) appropriate statistical approaches. Taken together, the available data is insufficient to draw any solid conclusions. More research in metabolomics with large sample sizes, rigorous data collection and appropriate statistical methods, and across various populations are warranted to determine if metabolomics will result in new indicators for the diagnosis, management, and prognosis of GDM and related complications.



## Appendix A. Literature search details.

|                   |  |   |
|-------------------|--|---|
| 1. Population     | Women with GDM during pregnancy (gestational diabetes mellitus or GDM)                       | ("diabetes, gestational"[MeSH Terms] OR ("diabetes"[All Fields] AND "gestational"[All Fields]) OR "gestational diabetes"[All Fields] OR ("gestational"[All Fields] AND "diabetes"[All Fields] AND "mellitus"[All Fields]) OR "gestational diabetes mellitus"[All Fields]) OR GDM[All Fields]  |
| 2. Exposure       | Metabolomics (metabolomics or metabolome)  | ('metabolomics'[MeSH Terms] OR 'metabolomics'[All Fields]) OR ('metabolome'[MeSH Terms] OR 'metabolome'[All Fields])  |
| 3. Outcome        | Type 2 Diabetes at follow-up   | "diabetes mellitus"[MeSH Terms] OR ('diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'diabetes mellitus'[All Fields] OR 'diabetes'[All Fields] OR "type 2 diabetes"[All Fields] OR 'metabolic diseases'[MeSH Terms] OR 'insulin resistance'[All Fields] OR "insulin sensitivity"[All Fields] OR 'metabolic syndrome x'[MeSH Terms] OR 'metabolic syndrome'[All Fields] OR 'HOMA-IR'[All Fields] OR "HOMA- beta"[All Fields] OR 'impaired glucose'[All Fields]  |
| 4. Outcome        | Children Obesity (obese OR overweight OR body mass index OR BMI OR body weight OR adiposity) | (((((('obesity'[MeSH Terms] OR 'obesity'[All Fields] OR "obese"[All Fields]) OR ('overweight'[MeSH Terms] OR 'overweight'[All Fields])) OR ("body mass index"[MeSH Terms] OR ("body"[All Fields] AND "mass"[All Fields] AND "index"[All Fields]) OR 'body mass index'[All Fields])) OR BMI[All Fields]) OR ('body weight'[MeSH Terms] OR ('body'[All Fields] AND "weight"[All Fields]) OR 'body weight'[All Fields])) OR ('adiposity'[MeSH Terms] OR 'adiposity'[All Fields])) OR (((('cohort studies'[MeSH Terms] OR ("cohort"[All Fields] AND "studies"[All Fields]) OR 'cohort studies'[All Fields] OR ('cohort'[All Fields] AND "study"[All Fields]) OR 'cohort study'[All Fields]) OR ("longitudinal studies"[MeSH Terms] OR ("longitudinal"[All Fields] AND 'studies'[All Fields]) OR 'longitudinal studies'[All Fields])) OR ('prospective studies'[MeSH Terms] OR ("prospective"[All Fields] AND 'studies'[All Fields]) OR 'prospective studies'[All Fields] OR ('prospective'[All Fields] AND 'study'[All Fields]) OR "prospective study"[All Fields])) OR (hazard[All Fields] AND ("Ratio (Oxf)"[Journal] OR "ratio"[All Fields]))  |
| 6. Study subjects | Human Study  |   |
| 1 + 2 + 4 + 6 + 7 | Human cohort   | (((((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields] OR GDM[All Fields]) AND (('metabolomics'[MeSH Terms] OR 'metabolomics'[All Fields]) OR ('metabolome'[MeSH Terms] OR 'metabolome'[All Fields])) AND ('diabetes mellitus'[MeSH Terms] OR ('diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'diabetes mellitus'[All Fields] OR 'diabetes'[All Fields] OR 'type 2 diabetes'[All Fields] OR 'metabolic diseases'[MeSH Terms] OR 'insulin resistance'[All Fields] OR 'insulin sensitivity'[All Fields] OR 'metabolic syndrome x'[MeSH Terms] OR 'metabolic syndrome'[All Fields] OR 'HOMA-IR'[All Fields] OR "HOMA- beta"[All Fields] OR 'impaired glucose'[All Fields])) AND (((('cohort studies'[MeSH Terms] OR ('cohort'[All Fields] AND 'studies'[All Fields]) OR 'cohort studies'[All Fields] OR ('cohort'[All Fields] AND 'study'[All Fields]) OR 'cohort study'[All Fields]) OR ('longitudinal studies'[MeSH Terms] OR ('longitudinal'[All Fields] AND 'studies'[All Fields]) OR 'longitudinal studies'[All Fields])) OR ('prospective studies'[MeSH Terms] OR ('prospective'[All Fields] AND 'studies'[All Fields]) OR 'prospective studies'[All Fields] OR ('prospective'[All Fields] AND 'study'[All Fields]) OR 'prospective study'[All Fields])) OR (hazard[All Fields] AND ('Ratio (Oxf)'[Journal] OR 'ratio'[All Fields])) AND "humans"[MeSH Terms]   |
| 1 + 2 + 4 + 6     | Cohort   | (((((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields] OR GDM[All Fields]) AND ("gastrointestinal microbiome"[MeSH Terms] OR 'gastrointestinal microbiome'[All Fields] AND "microbiome"[All Fields]) OR 'gastrointestinal microbiome'[All Fields] OR 'gastrointestinal microbiota'[All Fields] AND "microbiota"[All Fields]) OR "gastrointestinal microbiota"[All Fields])) AND ((((((('obesity'[MeSH Terms] OR 'obesity'[All Fields] OR 'obese'[All Fields]) OR ('overweight'[MeSH Terms] OR 'overweight'[All Fields]) OR ('body mass index'[MeSH Terms] OR ('body'[All Fields] AND 'mass'[All Fields] AND 'index'[All Fields]) OR 'body mass index'[All Fields]) OR BMI[All Fields]) OR ('body weight'[MeSH Terms] OR ('body'[All Fields] AND 'weight'[All Fields]) OR 'body weight'[All Fields])) OR ('adiposity'[MeSH Terms] OR 'adiposity'[All Fields])) AND (((('cohort studies'[MeSH Terms] OR ('cohort'[All Fields] AND 'studies'[All Fields]) OR 'cohort studies'[All Fields] OR ('cohort'[All Fields] AND 'study'[All Fields]) OR 'cohort study'[All Fields]) OR ('longitudinal studies'[MeSH Terms] OR ('longitudinal'[All Fields] AND 'studies'[All Fields]) OR 'longitudinal studies'[All Fields])) OR ('prospective studies'[MeSH Terms] OR ('prospective'[All Fields] AND 'studies'[All Fields]) OR 'prospective studies'[All Fields] OR ('prospective'[All Fields] AND 'study'[All Fields]) OR 'prospective study'[All Fields])) OR (hazard[All Fields] AND ('Ratio (Oxf)'[Journal] OR 'ratio'[All Fields]))                          |
| 1 + 3 + 4 + 6 + 7 | Human cohort   | (((((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields] OR GDM[All Fields]) AND ("gastrointestinal microbiome"[MeSH Terms] OR 'gastrointestinal microbiome'[All Fields] AND "microbiome"[All Fields]) OR 'gastrointestinal microbiome'[All Fields] OR 'gastrointestinal microbiota'[All Fields] AND "microbiota"[All Fields]) OR "gastrointestinal microbiota"[All Fields])) AND ((((((('obesity'[MeSH Terms] OR 'obesity'[All Fields] OR 'obese'[All Fields]) OR ('overweight'[MeSH Terms] OR 'overweight'[All Fields]) OR ('body mass index'[MeSH Terms] OR ('body'[All Fields] AND 'mass'[All Fields] AND 'index'[All Fields]) OR 'body mass index'[All Fields]) OR BMI[All Fields]) OR ('body weight'[MeSH Terms] OR ('body'[All Fields] AND 'weight'[All Fields]) OR 'body weight'[All Fields])) OR ('adiposity'[MeSH Terms] OR 'adiposity'[All Fields])) AND (((('cohort studies'[MeSH Terms] OR ('cohort'[All Fields] AND 'studies'[All Fields]) OR 'cohort studies'[All Fields] OR ('cohort'[All Fields] AND 'study'[All Fields]) OR 'cohort study'[All Fields]) OR ('longitudinal studies'[MeSH Terms] OR ('longitudinal'[All Fields] AND 'studies'[All Fields]) OR 'longitudinal studies'[All Fields])) OR ('prospective studies'[MeSH Terms] OR ('prospective'[All Fields] AND 'studies'[All Fields]) OR 'prospective studies'[All Fields] OR ('prospective'[All Fields] AND 'study'[All Fields]) OR 'prospective study'[All Fields])) OR (hazard[All Fields] AND ('Ratio (Oxf)'[Journal] OR 'ratio'[All Fields])) AND 'humans'[MeSH Terms] |
| 1 + 3 + 4 + 6     | Cohort   | (((((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields] OR GDM[All Fields]) AND ("gastrointestinal microbiome"[MeSH Terms] OR 'gastrointestinal microbiome'[All Fields] AND "microbiome"[All Fields]) OR 'gastrointestinal microbiome'[All Fields] OR 'gastrointestinal microbiota'[All Fields] AND "microbiota"[All Fields]) OR "gastrointestinal microbiota"[All Fields])) AND ('diabetes mellitus'[MeSH Terms] OR ('diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'diabetes mellitus'[All Fields] OR 'diabetes'[All Fields] OR 'type 2 diabetes'[All Fields] OR 'metabolic diseases'[MeSH Terms] OR 'insulin resistance'[All Fields] OR 'insulin sensitivity'[All Fields] OR 'metabolic syndrome x'[MeSH Terms] OR 'metabolic syndrome'[All Fields] OR 'HOMA-IR'[All Fields] OR "HOMA- beta"[All Fields] OR 'impaired glucose'[All Fields])) AND (((('cohort studies'[MeSH Terms] OR ('cohort'[All Fields] AND 'studies'[All Fields]) OR 'cohort studies'[All Fields] OR ('cohort'[All Fields] AND 'study'[All Fields]) OR 'cohort study'[All Fields]) OR ('longitudinal studies'[MeSH Terms] OR ('longitudinal'[All Fields] AND 'studies'[All Fields]) OR 'longitudinal studies'[All Fields])) OR ('prospective studies'[MeSH Terms] OR ('prospective'[All Fields] AND 'studies'[All Fields]) OR 'prospective studies'[All Fields] OR ('prospective'[All Fields] AND 'study'[All Fields]) OR 'prospective study'[All Fields])) AND 'humans'[MeSH Terms]   |

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|               |           |  |
|---------------|-----------|--|
|               |           | Terms] OR 'metabolic syndrome'[All Fields] OR 'HOMA-IR'[All Fields] OR "HOMA- beta"[All Fields] OR 'impaired glucose'[All Fields])   |
| 1 + 3 + 4 + 7 | Human     | ((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields]) OR GDM[All Fields]) AND ('gastrointestinal microbiome'[MeSH Terms] OR ('gastrointestinal'[All Fields] AND 'microbiome'[All Fields]) OR 'gastrointestinal microbiome'[All Fields] OR ('gastrointestinal'[All Fields] AND 'microbiota'[All Fields]) OR 'gastrointestinal microbiota'[All Fields])) AND ('diabetes mellitus'[MeSH Terms] OR ('diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'diabetes mellitus'[All Fields] OR 'diabetes'[All Fields] OR 'type 2 diabetes'[All Fields] OR 'metabolic diseases'[MeSH Terms] OR 'insulin resistance'[All Fields] OR 'insulin sensitivity'[All Fields] OR 'metabolic syndrome x'[MeSH Terms] OR 'metabolic syndrome'[All Fields] OR 'HOMA-IR'[All Fields] OR "HOMA- beta"[All Fields] OR 'impaired glucose'[All Fields]) AND 'humans'[MeSH Terms])     |
| 1 + 2 + 5     | All study | ((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields] OR GDM[All Fields]) AND (('metabolomics'[MeSH Terms] OR 'metabolomics'[All Fields] OR 'metabolome'[MeSH Terms] OR 'metabolome'[All Fields])) AND (((('obesity'[MeSH Terms] OR 'obesity'[All Fields] OR 'obese'[All Fields]) OR ('overweight'[MeSH Terms] OR 'overweight'[All Fields]) OR ('body mass index'[MeSH Terms] OR ('body'[All Fields] AND 'mass'[All Fields] AND 'index'[All Fields]) OR 'body mass index'[All Fields]) OR BMI[All Fields]) OR ('body weight'[MeSH Terms] OR ('body'[All Fields] AND 'weight'[All Fields]) OR 'body weight'[All Fields]) OR ('adiposity'[MeSH Terms] OR 'adiposity'[All Fields])) AND 'humans'[MeSH Terms])   |
| 1 + 2 + 5 + 7 | Human     | ((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields] OR GDM[All Fields]) AND (('metabolomics'[MeSH Terms] OR 'metabolomics'[All Fields] OR 'metabolome'[MeSH Terms] OR 'metabolome'[All Fields])) AND (((('obesity'[MeSH Terms] OR 'obesity'[All Fields] OR 'obese'[All Fields]) OR ('overweight'[MeSH Terms] OR 'overweight'[All Fields]) OR ('body mass index'[MeSH Terms] OR ('body'[All Fields] AND 'mass'[All Fields] AND 'index'[All Fields]) OR 'body mass index'[All Fields]) OR BMI[All Fields]) OR ('body weight'[MeSH Terms] OR ('body'[All Fields] AND 'weight'[All Fields]) OR 'body weight'[All Fields]) OR ('adiposity'[MeSH Terms] OR 'adiposity'[All Fields])) AND 'humans'[MeSH Terms])   |
| 1 + 3 + 5     | All study | ((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields] OR GDM[All Fields]) AND ('gastrointestinal microbiome'[MeSH Terms] OR ('gastrointestinal'[All Fields] AND 'microbiome'[All Fields]) OR 'gastrointestinal microbiome'[All Fields] OR ('gastrointestinal'[All Fields] AND 'microbiota'[All Fields]) OR 'gastrointestinal microbiota'[All Fields])) AND (((('obesity'[MeSH Terms] OR 'obesity'[All Fields] OR 'obese'[All Fields]) OR ('overweight'[MeSH Terms] OR 'overweight'[All Fields]) OR ('body mass index'[MeSH Terms] OR ('body'[All Fields] AND 'mass'[All Fields] AND 'index'[All Fields]) OR 'body mass index'[All Fields]) OR BMI[All Fields]) OR ('body weight'[MeSH Terms] OR ('body'[All Fields] AND 'weight'[All Fields]) OR 'body weight'[All Fields]) OR ('adiposity'[MeSH Terms] OR 'adiposity'[All Fields])) AND 'humans'[MeSH Terms]) |
| 1 + 3 + 5 + 7 | Human     | ((('diabetes, gestational'[MeSH Terms] OR ('diabetes'[All Fields] AND 'gestational'[All Fields]) OR 'gestational diabetes'[All Fields] OR 'gestational'[All Fields] AND 'diabetes'[All Fields] AND 'mellitus'[All Fields]) OR 'gestational diabetes mellitus'[All Fields] OR GDM[All Fields]) AND ('gastrointestinal microbiome'[MeSH Terms] OR ('gastrointestinal'[All Fields] AND 'microbiome'[All Fields]) OR 'gastrointestinal microbiome'[All Fields] OR ('gastrointestinal'[All Fields] AND 'microbiota'[All Fields]) OR 'gastrointestinal microbiota'[All Fields])) AND (((('obesity'[MeSH Terms] OR 'obesity'[All Fields] OR 'obese'[All Fields]) OR ('overweight'[MeSH Terms] OR 'overweight'[All Fields]) OR ('body mass index'[MeSH Terms] OR ('body'[All Fields] AND 'mass'[All Fields] AND 'index'[All Fields]) OR 'body mass index'[All Fields]) OR BMI[All Fields]) OR ('body weight'[MeSH Terms] OR ('body'[All Fields] AND 'weight'[All Fields]) OR 'body weight'[All Fields]) OR ('adiposity'[MeSH Terms] OR 'adiposity'[All Fields])) AND 'humans'[MeSH Terms]) |

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